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(54) **Lyophilized hydrocolloid foam.**

(57) A lyophilized foam sponge product having medically useful hemostatic and adhesive properties formed from the hydrocolloids, gelatin, pectin, and sodium carboxymethylcellulose and having a density of from about 0.01 to about 0.10 grams/cc. The gelatin is present at from about 20% to about 80% by weight of the final product and the pectin and sodium carboxymethylcellulose are each present at from about 10% to about 50% by weight of the final product. The product is prepared by forming an aqueous colloidal dispersion of hydrocolloids, aerating or foaming, freezing, and lyophilizing.

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LYOPHILIZED HYDROCOLLOID FOAM

This invention is directed to a medically useful lyophilized foam sponge product prepared from a mixture of gelatin, pectin, and sodium carboxymethylcellulose and having a density of from about 0.01 to about 0.10 grams/cc. The pectin and sodium carboxymethylcellulose are each present at from about 10% to about 50% by weight of the final product and the gelatin is present at from about 20% to about 80% by weight of the final product.

This invention is also directed to the method of preparing this lyophilized foam sponge product. This method includes dry blending the gelatin, pectin, and sodium carboxymethylcellulose, adding the mixture to water with agitation so as to form a colloidal dispersion having a solids content of from about 1% to about 20% by weight, foaming the colloidal dispersion so that its volume increases from about 10% to about 600%, freezing, and then freeze drying.

The product of this invention is a white spongy foam material whose characteristics vary somewhat depending upon the composition and processing techniques. The composition and method of preparation, which are explained in detail below, permit the product to be obtained in the form of a sheet which can be sliced or cut

to a desired size or milled into a granular form. The product can also be cast into discrete shapes such as cones, tampons, suppositories, etc.

The lyophilized product of this invention is capable of absorbing and holding many times its weight of whole blood or body exudates. The product is also bioabsorbable. Thus, it can be employed as a hemostatic agent to control visceral bleeding in areas of the body such as the pancreas, liver, or kidney where conventional means of control are technically impractical. The product can also be employed as an absorbable sponge in surgical procedures and for supportive surgical uses. The product can also be employed in the treatment of various open wounds such as decubitus and varicose ulcers.

The lyophilized foam product because of its hydrocolloid composition possesses wet-tack. Thus, the product can be employed as a bioabsorbable tissue adhesive for surgical procedures involving non-suturable tissue and in burn treatment and also as an adhesive in skin grafting procedures.

The solubility and absorbability of the lyophilized foam product can be reduced by cross-linking either before or after the lyophilization step. For some medical uses it may be preferable to have a partially insoluble product, for example in the adhesion of tissues, and in other medical uses a practically insoluble product may be preferred, for example when the product is shaped

to be used as a colostomy plug.

As a result of the unique combination of hemostatic, adhesive, bioabsorbability, and physical characteristics of the lyophilized foam product of this invention, it can be employed in numerous medicinal and veterinary procedures.

The lyophilized foam sponge product of this invention is formed from a mixture of gelatin, pectin, and sodium carboxymethylcellulose. The pectin and sodium carboxymethylcellulose are each present at from about 10% to about 50% by weight of the final product and the gelatin is present at from about 20% to about 80% by weight of the final product. In general, as the amount of gelatin is increased the final product becomes more pliable. The foam sponge product will have a density of from about 0.01 to about 0.1 grams/cc.

Preferably the pectin and sodium carboxymethylcellulose will each be present at from about 15% to about 35% by weight of the final product and the gelatin will be present at from about 30% to about 70% by weight of the final product.

The foam sponge product of this invention may be prepared as follows. There is first formed a dry blend of gelatin, pectin, and sodium carboxymethylcellulose. Preferably, these materials are comminuted to a finely divided form so as to aid in their mixing and increase the rate at which they will hydrate. Of course, these materials as well as any of the

other materials described below that may be employed in this invention should be of a pharmaceutically acceptable purity. The dry blend is then added with agitation to water to form a colloidal dispersion. Any conventional mixing device having a propeller, gate mixer, or homomixer can be employed. The amount of water and dry blend are controlled so that the initial dispersion has a solids content of from about 1% to about 20% by weight. The density and toughness of the final product will vary depending upon the solids content and the degree of aeration or foaming. Thus, the product obtained from a colloidal dispersion having a solids content of about 1% by weight will be relatively fluffy and soft whereas a product obtained from a colloidal dispersion having a solids content of about 5% or greater aerated or foamed to the same extent will be more rigid and tough. Preferably, the colloidal dispersion contains from about 3% to about 9% by weight of the dry blend of gelatin, pectin, and sodium carboxymethylcellulose and will result in a final product having a density of from about 0.01 to about 0.03 grams/cc. depending upon the degree of aeration or foaming.

In order to obtain a uniform product, the aqueous colloidal dispersion is aerated or foamed prior to freezing. The aeration or foaming increases the volume of the dispersion to about 10% to about 600% of the original volume. The presence of gas bubbles such as air or carbon dioxide prevents or at least retards depending upon the extent of entrainment the formation of patterns in the lyophilized

final product. Patterns are the result of ice crystal lattices forming in the dispersion during the freezing step and if present in the final product they are a source of nonuniformity and can cause mechanical weakness. Gas entrainment can be performed by whipping the dispersion and/or by means of a tube having a fitted cylinder that injects air or other gas into the dispersion. Dry ice can be used to generate carbon dioxide in the dispersion. Before freezing the aerated or foamed colloidal dispersion should contain from approximately 10% to about 85% by volume of entrapped gas, preferably about 60% by volume.

The foamed or aerated aqueous colloidal dispersion can be prepared at room temperature. Elevated temperatures could be employed to ensure dispersal of the hydrocolloids.

A surface tension modifier such as sodium hexametaphosphate or natural or synthetic surfactants such as lecithin and polyoxyethylene derivatives of sorbitan fatty acid esters such as Tween 60 can be added to the colloidal dispersion to stabilize the gas suspension and enhance the quality of the foam. Such agents can be added in varying amounts depending upon their surfactant ability but in general will vary from about 10% to about 100% by weight of the solids already present in the colloidal dispersion.

The foamed or aerated colloidal dispersion is then poured into metal or plastic containers and

frozen. The rate of heat transfer is important since at low levels of gas entrainment, if the dispersion is frozen too slowly, the gas bubbles may rise to the surface causing nonuniformity. In order to minimize variations in the freezing step, it is preferred that small containers be employed and that freezing step be performed in a well circulated cold room that is kept at about -20° C. The frozen material is then lyophilized in a conventional freeze drying apparatus at less than about 20°C and a vacuum of about 50 to 150 microns of Hg. After drying has been completed, the foam sponge product is maintained in a dry atmosphere (relative humidity less than 50%) to prevent condensation of moisture. The foam sponge product can then be cut into the desired size, shape and thickness and hermetically sealed in a plastic bag or glass container. The packaged product can be terminally sterilized by gamma radiation of about 1.5 mega rad.

Of course, if desired, the foamed aqueous colloidal dispersion can be freeze dried in a mold tray so to obtain the final product having a particular shape. Alternatively, a granular final product can be obtained by passing the dried foam sponge product through a screen before packaging. Preferably, a number 16 mesh screen is employed so as to obtain a granular product having a particle size of less than one mm.

The lyophilized hydrocolloid foam product can be cross-linked so as to reduce its solubility and absorbability. For example, a solution of a cross-linking agent such as formaldehyde, glutaraldehyde, alum or tannin can be added to the aerated or foamed colloidal dispersion at from about 0.1% to about 10% by weight of the combined gelatin, pectin, and sodium carboxymethylcellulose. Alternatively, the product can be cross-linked after the lyophilization step by exposing the freeze dried product to formaldehyde or glutaraldehyde vapor or ultraviolet radiation. As the amount of cross-linking increases the solubility of the product decreases.

Various pharmaceutically active compounds such as antimicrobial agents can also be added to aerated or foamed colloidal dispersion. In particular, where the product is intended for use as a hemostatic agent or surgical sponge, thrombin or other hemostatically useful substances can be added directly to the aerated or foamed colloidal dispersion.

Other substances can also be added to the aerated or foamed colloidal suspension. Plasticizers such as propylene glycol or glycerine can be included within the colloidal dispersion at up to about 30% by weight of the combined gelatin, pectin, and sodium carboxymethylcellulose. The addition of a plasticizing agent will enhance the flexibility and strength of the final product.

As discussed above, the foam sponge products of this invention have a density of from about 0.01 to 0.10 gm./cc. depending upon the weight percent of gelatin, pectin, and sodium carboxymethylcellulose. The foam sponge products of this invention can absorb from about 350% to about 900% of their own weight of heparinized whole blood and from about 700% to about 1500% of their own weight of water.

The water absorption rate of the foam sponge products of this invention are tested by placing a 0.75 inch by 0.75 inch piece on a sintered glass filter attached with a graduate pipet. The time required to have 0.05 ml. of water absorbed is found to be from about 30 to 100 seconds for the foam sponge product in which no cross-linking agent is present and from about 100 to 150 seconds for the cross-linked foam sponge product.

The adhesive strength of the foam sponge products of this invention are tested by sandwiching a pice of the product between two strips of pre-soaked dialyzer tubing, .875 inch wide and two inches long, loaded with a 50 gm. weight for three minutes. These two strips adhered by the foam sponge product are pulled apart by Chatillon guage and at 1.2 cm./min. the break point is registered. According to this procedure the break point on the Chatillon gauge is from about 400 to 900 gms.

The following examples are illustrative of the invention.

Example 1

A dry blend is formed consisting of 5 g. of sodium carboxymethylcellulose (extra fine), 5 g. of gelatin (Type A, high bloom, U.S.P. 100 mesh) and 5 g. of pectin (200 mesh). The mixture is passed twice through an 80 mesh screen. This dry blend is then slowly added to 500 ml. of water with vigorous agitation and a stream of air is blown into the bottom of the dispersion through a capillary tube. After approximately one hour, the colloidal dispersion becomes milky white and its volume increases to about 650 ml. The foamed dispersion is then poured into a 30 cm. by 45 cm. flat bottom metal tray and is frozen in a -20°C. cold room. After the dispersion is frozen solid the tray is transferred to a lyophilizer and the material is dried at -5°C. and 150 microns of Hg. After about 48 hours, the material is totally dried and it is removed from the lyophilizer and sliced to the desired size. The foam sponge product is then hermetically sealed inside a plastic bag or glass container and sterilized by gamma radiation at 1.5 Mrads.

This foam sponge product has a density of 0.04 gm/cc. and a pH of 4.5 ± 0.3 which is determined by dissolving 0.1 g. in 10 ml. of water.

Example 2

Sixty grams of a dry powder consisting of 30 g. of gelatin (Type B, low bloom, USP 100 mesh), 15 g. of sodium carboxymethylcellulose (fine) and 15 g. of citrus pectin (200 mesh) is rapidly added to 1 liter of purified water with vigorous agitation such as that produced by a ballon whip. Whipping is continued for approximately 10-15 minutes or until the volume of aerating foam is approximately 3 liters. The foam is then transferred to shallow pans (e.g., 45 cm. x 45 cm. x 1 cm.) or molds and is frozen at -5° to -20°C for approximately six hours. The frozen material is then lyophilized at 50 to 100 microns for approximately 36 hours at 20°C. The density of the resulting flexible foam product is 0.02 gm/cc.

The material can be sliced into a desired size and hermetically packaged. If desired, the product can be sterilized by exposure to gamma radiation at 1.5 Mrads.

Example 3

Following the procedure of Example 2 but employing as the powder a mixture of 54 g. of gelatin (Type A, high bloom, fine mesh), 18 g. of sodium carboxymethyl cellulose (fine), and 18 g. of citrus pectin (200 mesh), a foam product that is less flexible than that of Example 2 is obtained. This product has a density of about 0.03 g/cc.

Example 4

Following the procedure of Example 2 but employing as the powder a mixture of 45 g. of gelatin (Type A, low bloom, fine mesh), 22.5 g. of sodium carboxymethylcellulose (fine), and 22.5 g. of citrus pectin (200 mesh), a foam product intermediate in flexibility to those of Examples 2 and 3 is obtained. This product has a density of about 0.03 g/cc.

Examples 5 - 20

Following the procedure of Example 1 or 2 but varying the materials as set forth below additional foam sponge products within the scope of the invention are obtained.

Ex.	DRY BLEND			Weight % of Dry Blend In the Aq. Dis.	Volume % of Gas In the Aq. Dis.	Weight % Plasticizer (glycerol) Relative to Dry Blend in Aq. Dis.
	Weight % Gelatin	Weight % Pectin	Weight % Na CMC			
5	60	20	20	6	65	--
6	50	25	25	6	65	15
7	80	10	10	6	65	--
8	70	15	15	9	50	--
9	60	25	15	8	60	--
10	60	15	25	8	65	--
11	50	30	20	9	65	--
12	50	20	30	9	65	--
13	50	20	30	6	65	--
14	30	30	40	3	35	--
15	40	30	30	4	40	--
16	40	30	30	6	65	--
17	50	25	25	3	85	--

Ex.	DRY BLEND			Weight % of Dry Blend In the Aq. Dis.	Volume % of Gas In the Aq. Dis.	Weight % Plasticizer (glycerol) Relative to Dry Blend in Aq.Dis.
	Weight % Gelatin	Weight % Pectin	Weight % Na CMC			
18	33.3	33.3	33.4	4.5	65	30
19	45	30	25	7	65	--
20	66	17	17	9	65	--

Example 21

Following the procedure of Example 3 but adding from about 1 to about 5 ml. of formalin solution (37% by weight of formaldehyde gas in water) to the aerating foam prior to freezing and lyophilizing results in a foam that is almost completely insoluble in water.

Similarly, the aerating foams of Examples 1, 2 and 4 to 20 can be treated with formaldehyde prior to freezing and lyophilizing so as to cross-link one or more of the hydrocolloids and decrease the solubility of the final product.

Alternatively, the products of Examples 1 to 20 can be cross-linked after lyophilization by placing the product in a closed vessel for about two hours on a porous platform above a reservoir containing formalin solution. The formalin is presaturated with calcium chloride to maintain relative humidity at about 30 to 35%.

Example 22

Lyophilized thrombin is dispersed in a small volume of water at a concentration of approximately 10,000 units per 30 ml. This thrombin dispersion is added to the aerating foam of Example 1 and gently mixed. The thrombin containing foam is immediately frozen and then lyophilized according to the procedure of Example 1.

Example 23

The following in vivo study was performed to evaluate the hemostatic and bioabsorbability of a sponge product of this invention as compared with

the commercially available product Gelfoam® (Upjohn).

Twelve New Zealand White rabbits weighing approximately 2.5 kg. and having ear tags for identification were used in the study. Each rabbit was anesthetized by intravenous injection of sodium pentobarbitol. The abdominal cavity was opened and the liver was exposed. An approximately 3 cm. thick slice of a lobe was incised, removed from the liver, and weighed. Immediately after incision, a piece of the sponge product prepared according to the procedure of Example 1 or Gelfoam was placed on the incisions of four rabbits each. Incisions of four additional rabbits were left uncovered to serve as control. The incision sites were observed for hemorrhage, and the blood loss from each animal was weighed upon clotting.

Upon cessation of bleeding, the test materials were left in place, the abdominal wall sutured, and the animals observed for survival. After ten days, all animals were necropsied, and the incision sites were examined for fate of the foam and any gross signs of tissue reaction. Mean blood loss from the group treated with the sponge product of Example 1 was compared to those of control and Gelfoam treated groups by Student's t test. Sections of liver at the incision sites were examined for histological changes.

Table 1 shows the individual weights of the blood loss and the slice of liver removed from each animal as well as their group mean values.

Incisions produced seepage of blood from the livers. The amount of blood loss was variable in the control and Gelfoam treated groups. Mean values of the blood loss were 6.10 $\pm$ 1.76 g. in the control, 6.08 $\pm$ 2.06 g. in the Gelfoam-treated, and 0.97 $\pm$ 0.2 g. in the group treated with the product of Example 1. The difference between the control or Gelfoam groups and the group treated with the product of Example 1 was significant ($P < 0.05$).

TABLE I

Group Number	Rabbit Number	Blood Loss (g.)	Liver Removed (g.)
I	13	1.3	5.2
	14	1.0	4.3
	9	1.2	4.7
	16	0.4	5.1
Sponge Product of Example I	$\bar{x}$	0.97 $\pm$ 0.20	4.82 $\pm$ 0.21
	I vs. III	$p<.05$	not significant
	I vs. II	$p<.05$	$p<.01$
II	17	4.3	7.2
	18	2.6 (Blood collection incomplete)	5.9
Gelfoam	19	5.4	5.9
	20	12.0	6.5
	$\bar{x}$	6.08 $\pm$ 2.06	6.38 $\pm$ 0.31
	II or III	not significant	not significant
III	5	10.6	4.0
	11	2.7	4.8
	1	7.1	7.3
	7	4.0	7.1
Control	$\bar{x}$	6.10 $\pm$ 1.76	5.80 $\pm$ 0.83

Table II presents the results of pathological evaluation of incision sites in the liver of each animal. No test or control animal died during the ten day observation period.

On necrospsy, none of the sponge product from Example 1 was observed in the peritoneal cavity of any rabbit. Absence of this material was further confirmed on histological examination of the healed hepatic incisions. In animals treated with Gelfoam, however, the test material was still intact, grossly clearly distinguishable from the liver tissue, and adhered to the incision sites. Microscopically, it could be seen as a pink (hematoxylin-eosin-stained proteinaceous material) sponge infiltrated with blood of exudate. Gelfoam, therefore, was not absorbed during the 10-day period.

Livers of all animals were found to have healed with the formation of a scar at the incision sites. Histological evaluation of the incision sites revealed proliferation of fibroblasts, formation of giant cells indicating early hepatic regeneration, and focal necrosis or suppurative inflammation in some cases. Hepatic incision sites in three of the control animals showed hemorrhages. No hemorrhages were seen in either of the treated groups. Healing of the hepatic incisions in the three groups was similar, and no tissue reaction ascribable to the test materials was observed in any animal.

Thus, under the conditions of this study, the product of Example 1 was found to have a greater hemostatic effect than Gelfoam, was completely absorbed within 10 days, and elicited no tissue reaction in the peritoneal cavity.

TABLE II

Group No./Animal No.	Test Material	Scar	Supparation	Giant Cells	Focal Necrosis	Focal Hemorrhage
Sponge Product of Example I						
I 9	-	+	-	+	-	-
I 13	-	+	-	+	-	-
I 14	-	+	-	+	-	-
I 16	-	+	+	+	+	-
Gelfoam						
II 17	+	+	-	+	-	-
II 18	+	+	-	+	+	-
II 19	+	+	-	+	-	-
II 20	+	+	+	+	-	-
Control						
III 1	-	+	-	+	-	-
III 5	-	+	-	+	+	+
III 7	-	+	-	+	-	+
III 11	-	+	-	+	+	+

- = absent + = present

CLAIMS

1. A medically useful lyophilized sponge product having a density of from about 0.01 to about 0.10 gm/cc, said sponge product comprising a mixture of the hydrocolloids, gelatin, pectin, and sodium carboxymethylcellulose, said pectin and sodium carboxymethylcellulose each being at from about 10% to about 50% by weight of the final product and said gelatin being present at from about 20% to about 80% by weight of the final product.

2. The product of Claim 1 wherein said pectin and sodium carboxymethylcellulose are each present at from about 15% to about 35% by weight of the final product and said gelatin is present at from about 30% to about 70% by weight of the final product.

3. The product of Claim 2 wherein said gelatin, pectin, and sodium carboxymethylcellulose are present in about the same amount by weight.

4. The product of Claim 3 having a density of about 0.04 gm/cc.

5. The product of Claim 2 wherein said gelatin is present at about 50% by weight and said pectin and sodium carboxymethylcellulose are each present at about 25% by weight.

6. The product of Claim 5 having a density of about 0.02 gm/cc.

7. The product of Claim 5 having a density of about 0.03 gm/cc.

8. The product of Claim 2 wherein said gelatin is present at about 60% by weight and said pectin and

sodium carboxymethylcellulose are each present at about 20% by weight.

9. The product of Claim 1 wherein a pharmaceutically active material is included within the sponge.

10. The product of Claim 9 wherein the pharmaceutically active material is thrombin.

European Patent Office

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Application number EP 81 30 2822

EUROPEAN SEARCH REPORT

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim
	US - A - 2 558 395 (A. STUDER) * Whole document *	1-10
	FR - A - 1 505 318 (E.R. SQUIBB) * Examples 7-11, summary *	1-8
	US - A - 3 972 328 (J.L. CHEN) * Claims *	1-9
A	US - A - 2 764 159 (J.N. MASCI et al.)	
A	US - A - 2 465 357 (J.T. CORRELL)	
A	US - A - 3 249 109 (H. MAETH)	
A	CHEMICAL ABSTRACTS, vol. 75, no. 6, August 9, 1971, page 276, abstract 40327s Columbus, Ohio, US K.BOLEWSKI et al. "Carboxymethyl cellulase symplex (Na-KMC) with gelatin" & FARM. POL. 1970, 26(10), 793-8	
E	EP - A - 0 026 572 (KINGSDOWN MEDICAL CONSULTANTS) * Page 5; claims *	1-9

CLASSIFICATION OF THE APPLICATION (Int Cl.³)

A 61 L 15/00
A 61 L 15/04

TECHNICAL FIELDS SEARCHED (Int. Cl.³)

A 61 L 15/00
15/01
15/04

CATEGORY OF CITED DOCUMENTS

X: particularly relevant
A: technological background
O: non-written disclosure
P: intermediate document
T: theory or principle underlying the invention
E: conflicting application
D: document cited in the application
L: citation for other reasons

&: member of the same patent family, corresponding document

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
The Hague	26-10-1981	LENSEN

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